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Sensitivity and Specificity of Various Serological Tests for Detection of *Brucella spp*. Infection in Male Goats and Sheep

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Abstract

Brucellosis is a zoonosis found throughout the world with major implications both in the field of public health and farming economy. It is most often caused by *Brucella abortus* and *Brucella melitensis*. This study aimed for evaluation of the sensitivity and specificity of immunochromatographic assay (ICA) and comparing the results with those obtained from Rose Bengal Plate Test (RBPT) and in direct Enzyme Linked Immunsorbent Assay (ELISA) for serodiagnosis of sheep and goat brucellosis. Out of 41 sera samples (21 ram and 20 male goats) which were examined, antibodies against *Brucella* were detected in ram 8 (38.09%), 6 (28.57%) and 16 (76.19%) in ram samples, whereas they were detected in 3 (15%), 1 (5%) and 12 (60%) serum sample of goats males by RBPT, (ICA) and iELISA respectively. The kappa value between iELISA and ICA was −0.08 and that between RBPT and ELISA was −0.01 in ram and the kappa value between iELISA and ICA was −0.06 and that between RBPT and ELISA was −0.14 in male goats. The sensitivity for RBPT and ICA were 37.5% and 25% while specificity was 60% and 60% in ram; whereas, in male goats, sensitivity for RBPT was 8.33% and ICA was 8.33% while specificity was 75% and 100% respectively. It can be concluded that the developed ICA is immunodiagnostic assay, and it is rapid, non-expensive, economical and suitable for large-scale screening in developing countries and rural areas.

Keywords

Brucellosis, RBPT, ICA, iELISA, Sheep, Goat

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1. Introduction

Brucellosis is an important zoonosis of wild and domestic animals in which man is an accidental host. It has a worldwide distribution, especially in Mediterranean countries and the Middle East and it remains a significant public health concern [1]. Abortion, placentitis, epididymitis and orchitis are the most common clinical manifestations in animals [2].

Brucella melitensis may also cause abortion in cattle, although it is mainly associated with sheep, goats and wildlife [3]. Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species. Human infection due to *Brucella* from camels is known to occur mostly through the consumption of unheated milk [4] [5].

Diagnosis of *Brucella spp*. infection is mainly based on the detection of antibodies in serum by serological test. The standard Rose Bengal Plate Test (RBPT) is used to detect antibodies against *Brucella abortus* and *Brucella melitensis* infections. This test has been useful in eradication of bovine brucellosis in some countries [6]. The enzyme linked immunosorbent assay (ELISA) is a highly specific and sensitive diagnostic assay since it directly detects antibody and has minimal or no false positive reactions of agglutination test [7].

The convenience and speed of the test have been achieved by a novel concept of ICA which is a simplified version of ELISA [8] [9]. The objective of this study was to assess the diagnostic value of the ICA device for serodiagnosis of ovine and caprine brucellosis and compare the results with those obtained from RBPT and iELISA.

2. Materials and Methods

Samples Collection

Forty-one (41) blood samples collection (21 ram and 20 male goats) from healthy apparently sheep and goat males herds, aged between 3 - 8 years old, through period from January to March 2015 in Abu-Graib region was done.

We transported blood samples to the laboratory inside the ice bag and centrifuged the blood samples at 3000 r.p.m./15 minute [10].

3. Serological Tests

3.1. Rose Bengal Plate Test (RBPT)

The serum samples and antigen were brought at room temperature from the freezer and refrigeration respectively; each serum sample (30 µl) was placed on an enamel plate, and added the same of Rose Bengal antigen to each serum and mixed by plastic rod for each test, agitated gently for 4 minutes on a rocker. After that the test was read immediately. Any visible agglutination was considered positive; this test was used as described by OIE (2004).

3.2. Immunochromatographic Assay (ICA)

The Anigen Rapid *B. melitensis* Ab Test Kit is a chromatographic immunoassay for the qualitative detection of *Brucella melitensis* antibody in the whole blood, plasma, serum and milk.

The Anigen Rapid *B. melitensis* has a letter T and C as “Test Line” and “Control Line” on the surface of the kit. Both the “Test Line” and “Control Line” in the result window are not visible be for applying any samples. The “Control Line” is used for procedural control. The control line should always appear if this procedure is performed properly, and the test reagents of the control line are working. A purple “Test Line” will be visible in the result window if there are enough *Brucella melitensis* antibodies in the specimen [11].

3.3. In Direct Enzyme Linked Immunsorbent Assay (iELISA)

The test was performed as described by the manufacturer.

3.4. Statistical Analysis

Data were statistically analyzed by using statistical package for social science SPSS [12]. The agreement between serological tests was calculated using kappa analysis.

Sensitivity = true positive/true positive + false negative.
Specificity = true negative/false positive + true negative.

4. Results

Out of 41 (21 sheep and 20 goats male) examined sera by using three tests including RBPT (**Figure 1**), ICA (**Figure 2**) and iELISA antibodies against brucellosis, 8 (38.09%), 6 (28.57%) and 16 (76.19%) in ram, whereas 3 (15%), 1 (5%) and 12 (60%) in goats males were positive with RBPT, ICA and iELISA respectively (**Table 1**).

Table 2 showed that the sensitivity for RBPT and ICA were 37.5% and 25% respectively, while specificity was 60% and 60% respectively in rams; whereas, in goat males sensitivity for RBPT and ICA was 8.33% and 8.33% respectively, while specificity was 75% and 100% respectively. There is agreement of RBPT (K = −0.01 and K = −0.14) and ICA (K = −0.08 and K = 0.06) in relation to iELISA in sheep and goats respectively (**Table 2**).

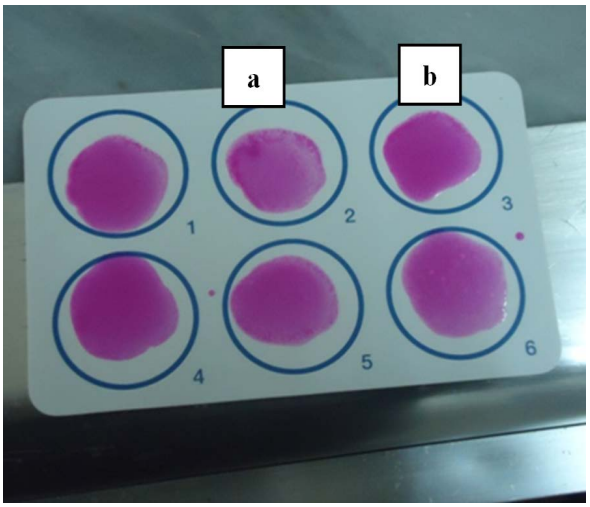

Figure 1. Rose Bengal plate test. (a) Positive result showing agglutination particles of antigen-antibody reaction; (b) Negative result: showing no agglutination.

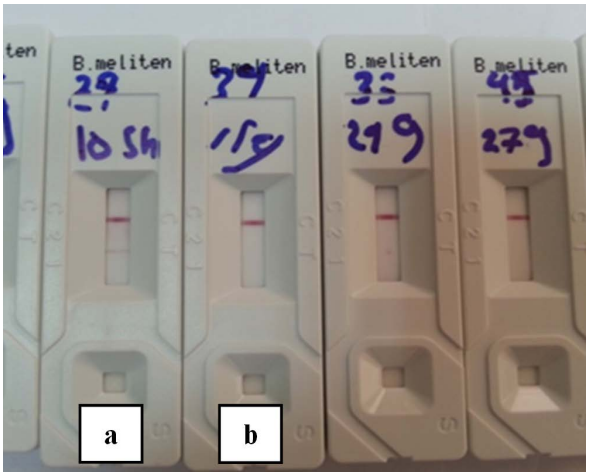

Figure 2. *Brucella* Immunochromatographic assay. (a) Positive result: showing two purple color bands in the result window; (b) Negative result: showing single purple color band in the result window.

Table 1. Comparison between (ICA) and other serological tests (RBPT and iELISA) in ram and goat males.

Animals	RBPT			ICA			iELISA		
	Positive	Negative	Percentage (%)	Positive	Negative	Percentage (%)	Positive	Negative	Percentage (%)
Ram	8	13	38.09	6	15	28.57	16	5	79.19
Goats	3	17	15	1	19	5	12	8	60

Table 2. The sensitivity and specificity of different serological tests compared with iELISA.

Animals	RBPT			ICA		
	Sensitivity	Specificity	Kappa	Sensitivity	Specificity	Kappa
Ram	37.5	60	−0.01	25	60	−0.08
Goat	8.33	75	−0.14	8.33	100	0.06

5. Discussion

RBPT was found to be more sensitive than ICA in sheep sera but ICA and RBPT were equal in specificity, while in goats RBPT and ICA were equal with sensitivity but ICA was more specific than RBPT as ICA can detect both IgG and IgM antibodies to *Brucella* in animals. The result is agreement with other studies [6] [13].

Although IgM is the first class of immunoglobulins appearing after infection, yet it was proved to be of non-specific nature, besides, most Gram negative bacteria produce IgM similar to those produced by *Brucellae* [10], Corbel [14]. Moreover, RBPAT detects mainly IgM and IgG1. Despite these limitations, the RBPAT may be used as a screening test to ascertain exposure of animals to infection due to *Brucella* species. So the conventional agglutination tests have good sensitivity but their lack of specificity and the occurrence of false positive make a specific test necessary [15].

There are differences between RBPT and other tests may be due to false positive results with RBPT. These results occur for a variety of many reasons which are found most prominent cross reactions with other bacteria including *Yersinia* enterocolitica 0:9, which share with *Brucella spp*. by major O-polysaccharide almost completely, also serological cross-reactions between smooth *Brucella spp*., *E-coli* O116: H21, O157: H7, *Francisella tularensis*, *Salmonella* serotypes of Kauffman-White group N, *Pseudomonas maltophilia* and Vibrio cholera [16] [17]. In addition to sensitivity and specifity of RBPT may affect by type of antigen and room temperature also most of the serological tests used were liable to radical change in their incidence; the great number of false positive detected by RBPT in the first examination was due to the activity of specific antibodies [18].

The (ICA) is easy and rapid to be done and it has several advantages that allow testing in the management of large numbers of serum samples without specialized training, expertise, electricity and nor expensive equipment and it used in the animals from developing countries and other migratory populations [19] [20]. Also these devices can storage without the need for cooling and the test results are obtained almost immediately and visual inspection with the naked eye [19]. Many scientific studies by Smits *et al*. [21]; Dey *et al*. [22] refer to that ICA is accurate as compared with the standard microscopic agglutination test (MAT) also the sensitivity and specificity of LAT are 88% and 98%, respectively [23].

It is clear that direct Enzyme linked immunosorbent Assay (ELISA) is a much better option for diagnosis of brucellosis rather than RBPT and ICA because has several advantages when compared with other test. Firstly, it is a direct method of detection of specific antibody and therefore, it is not prone to false positive reaction. Secondly, it is more sensitive than other agglutination tests and thus has the potential to detect infected animals. Thirdly, the antibody enzyme conjugate employed has light chain reactivity and thus is able to detect all classes of antibody. A combine determination of all classes of antibody allows accurate serological diagnosis at any stage of disease. Fourthly, ELISA results provide an epidemiological tool for investigating the infective status of flocks [24] [25]. In addition, the enzyme immunoassays are objective and easy to perform and may be automated to permit the processing of a large number of sera within a short time [26].

6. Conclusion

The high sensitivity rate is revealed by RBPT suggesting its use as screen test while higher specificity is revealed by ICA suggesting its use for confirmation of positive samples screened by RBPT; it could be used for confirmation of positive samples screened by RBPT. Additionally the ICA was considered simple, rapid, economical and suitable for large-scale screening in endemic areas. Also we found that iELISA was better than RBPT in sheep and goat brucellosis due to the ability to differentiate between acute and chronic stage of the disease.

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